

**Host-parasite relationships of *Uvulifer ambloplitis* in fish of Douglas
Lake**

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Abstract:

Uvulifer ambloplitis is a digenetic trematode that infects aquatic snails and freshwater fish as first and second intermediate hosts and kingfishers as definitive hosts. Parasitic infection in fish is often referred to as “black spot” for fish respond to the metacercaria by producing a black cyst around the infection. This study served as a preliminary survey of the infection rates of *U. ambloplitis* in fish in Douglas Lake. Fish were collected from three locations around Douglas Lake to investigate whether or not there is a variation among the percent of fish infected as well as the average load of the parasite per species. A significant difference was found for both percent of infection as well as number of spots per fish. However, it is unclear whether or not discrepancies related to infection of species is due to differential species susceptibility to infection or differing host responses to parasitism.

Introduction:

The parasite, *Uvulifer ambloplitis*, is a digenetic trematode of the family Strigeidae. *U. ambloplitis* has an indirect lifecycle involving three hosts, with its definitive host being limited to the Kingfisher (*Cerle alcyon*) in North America. Its lifecycle begins when unembryonated eggs are passed in the droppings of kingfishers, where within three weeks they hatch in the water. The miracidia then penetrate the snails of *Helisomatrivolvus* and/or *Helisomacompanulata* where they transform into mother sporocysts. These sporocysts are about 2 mm when they mature, and cercariae develop about 6 weeks after initial infection. When the mature fork-tailed cercaria are shed from the snail host they hang in the water until they come in contact with their next fish intermediate host. The cercaria then attaches to the fish and penetrates the skin. Once the cercaria become embedded in the host tissue, fish within a few hours secrete a cyst wall for protection from attack of the host's immune system. They transform into metacercariae and after one day a fibrous capsule is formed around the parasite. As a response to the metacercaria's encasement, the host deposits a black pigment around the cyst. Kingfishers subsequently become infected by eating infected fish. The adult parasites attach to the intestinal mucosa of the bird, where they mature and produce eggs within about 27 days. The cycle is perpetuated when they defecate in the water, further infecting snails and successive fish (Olsen, 1968; Lemly & Esch, 1984; Berra & Au, 1978).

Completion of the life cycle is therefore dependent on the feeding and foraging habits of kingfishers on infected fish as well as the prevalence of infective snails in their habitat. These factors must coexist in the same area and time for the parasite to propagate. The Belted Kingfisher, which is the species of kingfisher on Douglas Lake, has a range throughout most of

the United States and Canada and infection rate has been reported to as high as 74%. Its habitat is dependent on clear waters in which they forage for fish. In addition, Kingfishers preferentially select nests located near their foraging territory; however, they can be found up to a mile away from their feeding grounds (Brooks & Davis, 1987; Ivory, 1999). Kingfishers tend to reproduce from April to July and it has been found that their reproductive periods overlap with the maximum prevalence of snails and their infection rates. The highest snail abundance and shedding percentages usually occur in July and correlate with the foraging and reproductive activities of the kingfishers adding large numbers of parasitic eggs to the system in the early summer. Snails begin to shed in May and continue until August when young kingfishers fledge and disperse reducing parasitic inputs and infection rates. Snails subsequently become scarce from September to May (Lemly & Esch, 1984).

Kingfishers are about the size of a pigeon and their diet consists predominantly of any type of small fish they can fit in their beaks, although they have been known to prey on crayfish, frogs, tadpoles, and other aquatic organisms (Ivory, 1999). Therefore the persistence of *U. ambloplitis* is also limited to the size of fish that it infects. Larger fish infected with the metacercaria will not be passed on and propagate in the definitive host. In fact, it has been shown the survival of metacercaria in fish that have overwintered has been correlated with the size of the fish, with metacercaria in smaller fish having a higher survival rate than larger fish. Reasons for reduced parasite survival after wintering may be related to low temperature effects and or time-dependent attrition of the encysted metacercaria possibly due to better immune systems of larger fish. However, regardless of the proximate cause this characteristic, is important adaptive feature for successful completion of the parasites lifecycle, for smaller fish are more easily captured and

ingested by kingfishers and would increase the probability of transmission (Lemly & Esch, 1984). It has also been found that infection increases the metabolic requirements of fish resulting in a negative correlation between the intensity of parasitism and both body condition and lipid content of infected fish. This increases the mortality rate of overwintered fish that do not have enough energy reserves to maintain body functions.

The infection of the metacercaria, also known as black spot disease, has been reported for many species of freshwater fish. This study served as a preliminary survey of the infection rates of *U. ambloplitis* in Douglas Lake. It investigated whether or not there was variation between different species of fish and the percent of fish infected as well as the average load of the parasite per species. Because kingfishers will forage any type of fish within the size range of their beaks, this study investigated whether or not different species are more susceptible to parasitism by *U. ambloplitis*.

Materials and Methods

Fish samples were collected from three locations around Douglas Lake in Pelston, Michigan: Grapevine Point, Hook Point, and near the boat well of Lake Side Lab on the University of Michigan Biological Station's Campus. Sites were chosen based on witnessed presence of a kingfisher and or habitat quality that be presumed feeding grounds for kingfishers. Site one was located at Hook point on the north end of Fish Tail Bay. The habitat consisted of a sheltered bay of a sandy substrate with thick vegetation. A second site was located near the tip of Grapevine point where kingfishers were observed on multiple occasions. Traps were set out along a fallen tree into the water where kingfishers were seen perching. The lake bottom of this location was sandy with a thick silt layer of clay and some vegetation. The third site was located near the boat

well of Lake Side Lab. While no kingfishers were observed in this location, it was very similar to the Grapevine point with a fallen tree extending out into the water; however, the lake bottom consisted of a more rocky substrate rather than sand with some vegetation as well. Fish were collected using minnow traps. At each site, three lines of minnow traps were set out with three traps per line. Traps on each line were placed at three depths, the first at 50 cm, the second at 75 cm, and the third at 100 cm (Figure 1). Different depths were used to incorporate more fish and increase the number of species captured. Samples were collected every 24 hours.

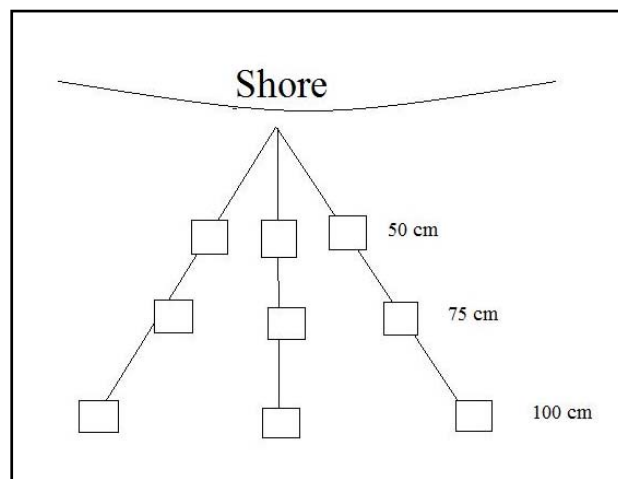


Figure 1: Layout of traps

Once collected, fish were weighted using a spring scale and the length, width (at the widest part), and height (not including fins) were measured using calipers. The number of spots were counted and all data were recorded.

A Chi-Square analysis was run as well to determine significance between percent infected and species of fish and because our data was non-parametric, a Kruskal-Wallis test was run to determine if there was a significant difference between number of black spots and species of fish.

Results:

As is evident from figure 2 there was a significant difference between species of fish and percent of that were infected ($\chi^2=34.24588$, $p=1.54922E-05$).

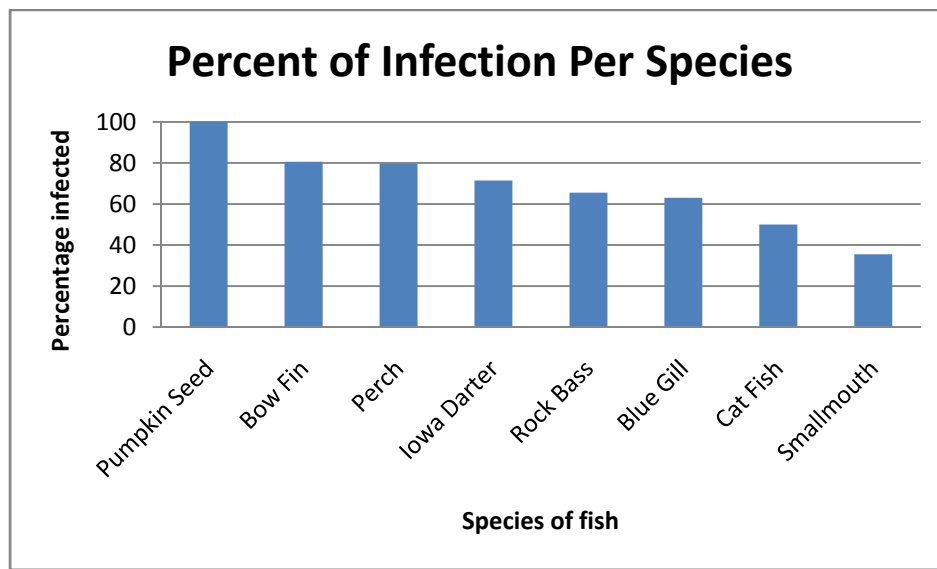


Figure 2: Percentage of Fish Infected Per Species of Fish

A significant difference was also found in the relative abundance (number of black spots) between different species of fish ($\chi^2=44.572$, $p=0.000$) (Figure 3).

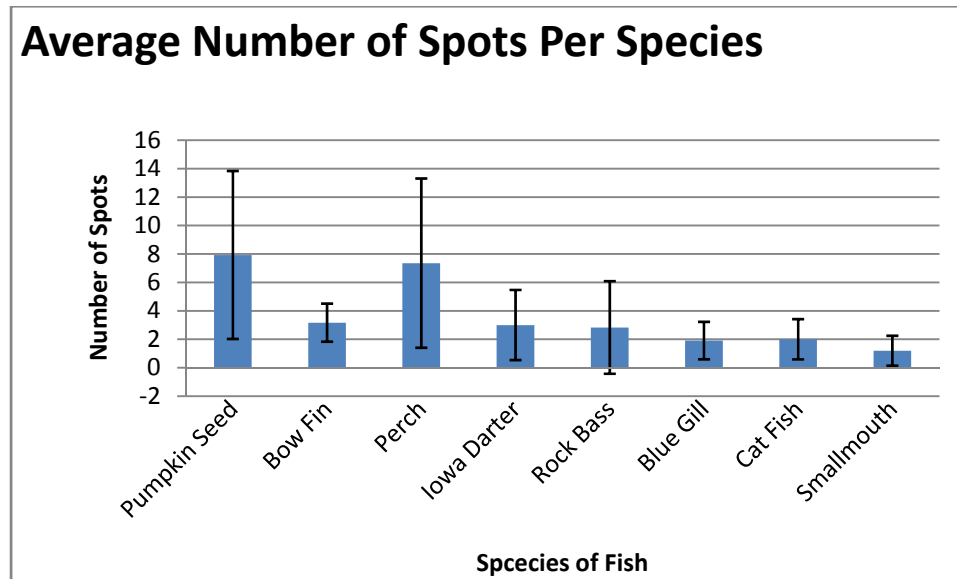


Figure 3: Average Number of Spots per Species

Discussion:

While results indicate that there is significant difference between both percent infected and intensity of infection, particular species of fish appear contribute more to the significance levels.

For example pumpkin seed, in both levels of statistical analysis, appear to be significantly different from other species. They have both the highest percentage of infection as well as highest average number of infections. However, the large range of infection may skew these results. While the average number of infections was 7.923 spots, the standard deviation was 11.8143 with infections ranging from 40 to 1. If one removed the largest outliers (25, 40 and 13), the average infection decreases to only 2.5, well within the range of other fish species.

Another species whose significance should also be discussed is the bowfin. All 36 bowfin collected were from the same trap on the same sampling day. The brood had an average infection rate of 3.166 with a standard deviation of 2.677. Next to pumpkin seed their percentage of infected fish was the second highest. Bowfins at a young age travel in schools so it makes sense that a brood of fish would have a uniform infection rate for they were all presumably exposed to the same levels of cercaria. This reasoning helps to explain the high percentage of infection as well as lower standard deviation. Nevertheless, it is important to point out that this brood may not be representative of their true infection rates; however, because only one brood of bowfins was collected there is no way to know unless further data is collected.

The finding that both percentage of infection and infection rates per species are both significantly different may imply that fish are differentially susceptible to *U. ambloplitis*. However, it is important to keep in mind that infected fish collected represent only the living fish infected with *U. ambloplitis*. It has been found that mortality rates of heavily infected fish are much higher than fish with either no or low infection rates, especially after overwintering. Therefore, higher percentages and incidences of black spot may be influenced by differential survivorship of species and their response to infection rather than their general susceptibility of parasitism.

While some controversy still exists concerning the effects of black spot infection on the body condition of fish, it has been found that parasite infection greatly increases the metabolic rate in the host bluegills, which subsequently depletes their energy reserves. A study by Lemly and Esch (1986) found that almost all heavily infected bluegills did not survive when exposed to cooling temperatures (below 10°C) as they would be during winter, with no significant difference when fish were held at a constant warmer temperature. Additionally non-infected to lightly infected

individuals had a higher percentage of survival after the winter. Bluegill mortality during winter is greatly influenced by their lipid content, which is subsequently reduced upon infection. When infection occurred prior to overwintering, parasitized fish had insufficient energy reserves to meet metabolic needs. This finding has been supported by both laboratory and field investigations, indicating a direct relationship between infection rates and both the changes in lipid content and mortality of *U. ambloplitis* (Lemly and Esch 1984). This reduction in survival due to infection may be represented in our data of bluegill and possibly other centrarchids; however, the effect on survival of other families of fish has not been extensively studied. Therefore, the lower average number of infections of fish may not represent differential infective susceptibility of the parasite, but rather differential host response to the parasite.

For example, perch are possibly less susceptible to mortality due to infection. They had both a high infection rate as well as total percent infected. It was also quantitatively noticed that perch tended to be of a larger size than most of the other fish with high infections. This may suggest that the host response of perch may not be as extreme. Possibly more perch are overwintering than other species of fish, and because spots do not disappear after infection, their total numbers of spots are compounded.

It is also important to note that number of black spots do not always correlate with infection rates, especially in fish that have overwintered. Our largest fish collected, that presumably experienced a winter season, tended to perch and pumpkinseed. These two species also had the highest infection rates and number of spots. However, as mentioned before, the metacercariae in larger fish have a lower overwinter survival rate. While the parasite may die, the black cyst may remain in the living fish. Therefore, fish that are less susceptible to lipid depletion due to

infection and therefore higher rates of survival may also have a larger number of “empty” black spots that are compounded with new infections the following summer. Therefore infection rates cannot be quantified by number of black spots in fish, especially those who have overwintered and survived (Lemly & Esch 1983).

Thus, some suggestions for future research include investigation and comparison of different species of fish in relation to their actual overwintered infection rates. While some studies exist on the relation of size of fish and metacercarial survival, there is little research in regards to species of fish and metacercarial survival. Furthermore, future studies may be able to use and expand on the data collected on effects of the depth of the fish caught and infection rate. Kingfishers can only dive to a limited depth to retrieve fish, so infection rates may decrease at certain depths. Also investigations of snails’ special distribution throughout Douglas Lake and their infection rates would also be relevant in further studies as well as more in-depth observations of behaviors of kingfishers. Future studies may want to look into comparing locations of nesting territories of kingfishers to areas non-inhabited to compare infection rates as well. Additionally, this study surveyed infection rates of fish in Douglas Lake over a two week period, providing only a snapshot view of seasonal infection. It would be interesting to see if there is a correlation to time of season and infection rate of fish when higher inputs of infected eggs are entering the system.

In conclusion, while this study found that there is a significant difference between percent of fish infected and number of infection per fish in different species there are numerous variables playing into infection rates. Therefore, more comprehensive data is needed to draw any definitive conclusions on the behavior and host response of *U.ambloplitis*.

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